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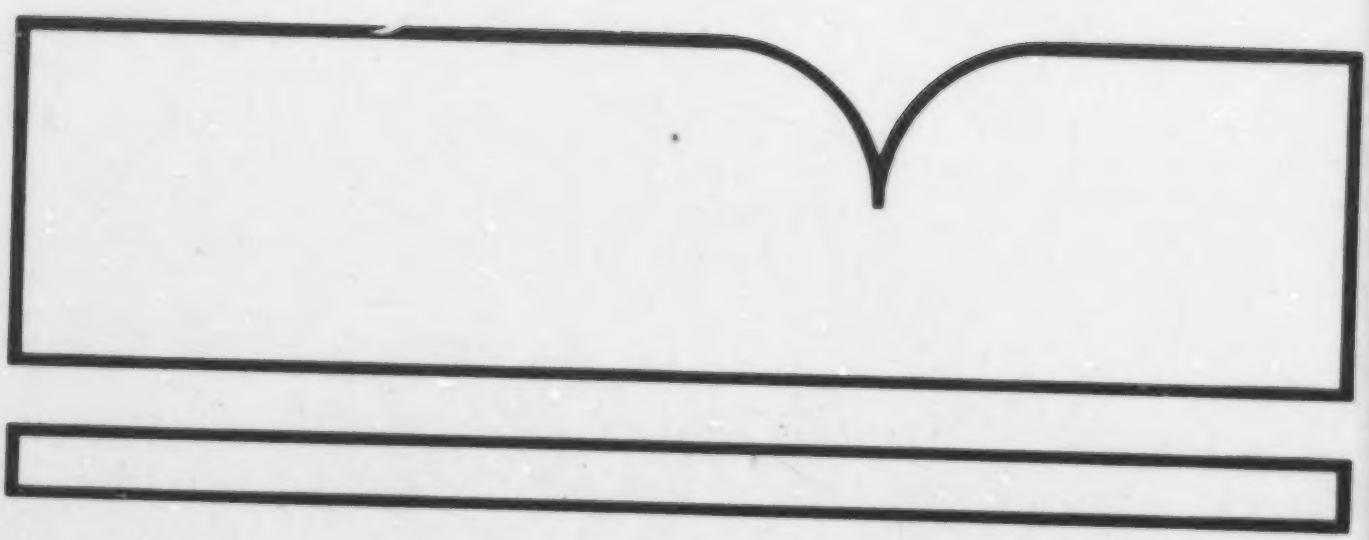

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16. ABSTRACT

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ATMOSPHERIC BENZENE DEPLETION BY SOIL MICROORGANISMS*

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Abstract. Gaseous benzene was rapidly depleted in exposure chambers containing viable soils and plants. When separate components of the system were analyzed, no benzene was detected in soils, plants, or water. Soil microorganisms were shown to be responsible for metabolizing benzene, yielding CO_2 as the main product. The rates were sufficiently rapid to suggest that this reaction forms a major pathway for the elimination of benzene from the environment.

1. Introduction

Detection of volatile organic carcinogens, including benzene, in the air of the United States (Mara and Lee, 1978) has drawn attention to the inadequacy of information describing the interactions and ultimate fate of the majority of these compounds in the environment (Bertke *et al.*, 1977; Cross *et al.*, 1979). For example, from emission data for benzene (Bertke *et al.*, 1977) it would be anticipated that environmental levels of this compound would be equivalent to or greater than those observed for the freons (I.M.O.S. task force, 1975) if the environmental stability previously reported (Mara and Lee, 1978) existed. Thus, fate and transport information related to major pathways of disappearance of benzene is important in order to assess the impact of this compound on the environment and man.

Benzene, as the simplest aromatic hydrocarbon, is the main substrate from which many compounds are synthesized. Consequently, it is commercially the organic chemical produced in the largest amount in the United States, and because it has a high vapor pressure (100 mm Hg at 26.1°C) it is lost to the atmosphere in large quantities (247×10^6 kg yr^{-1}) (Bertke *et al.*, 1977). Air concentrations are highly variable, especially near producer and user industries and are reported as ranging between a trace to as high as 50 ppm with many values being in the range of 0.05 to 0.5 ppm (Pilar and

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Graydon, 1973; Pellizari, 1976; Dorrigan *et al.*, 1976; Walker, 1976; Mara and Lee, 1978).

Benzene is considered to be quite stable in the atmosphere despite photolytic reactions (Howard and Durkin, 1974), and reaction with air oxidants (Bertke *et al.*, 1977; Matsuura and Omura, 1974). Metabolism of benzene has been demonstrated to occur by fungi and yeast (Gibson, 1971; Neujahr *et al.*, 1974; Evans, 1963, 1977; Rao and Bhat, 1971), soil microbes (Ribbons, 1966; Evans, 1977; Rogers *et al.*, 1980) and fruits (Durmishidze *et al.*, 1974). However, the information presented does not allow for a description of the environmental fate of benzene.

This research was undertaken to describe the dynamics which control the fate of atmospheric benzene when in contact with plants, soil, and water.

2. Methods

Experiments were conducted using exposure chambers designed to provide conditions that permit plants and soil organisms to function normally. After equilibration the contaminant – in this case benzene – was injected at a specific atmospheric concentration. Homogeneous air concentrations were observed within 1 minute of sample injection. The physiological parameters of transpiration and photosynthesis were monitored to assure normal plant metabolic functions.

A schematic diagram of the exposure chambers is presented in Figure 1. All structural materials were either glass, copper, or stainless steel. The humidity was controlled by circulating the air over a temperature controlled base plate. Evapotranspirational water condensed on the base plate and drained from the chamber. Subsequently it was sampled for the presence of benzene and degradation products. The base plate was maintained at 12 °C, which in the light, resulted in an atmospheric humidity of 42% and an average evapotranspirational water flow of 3.5 ml min^{-1} m^{-2} of soil surface

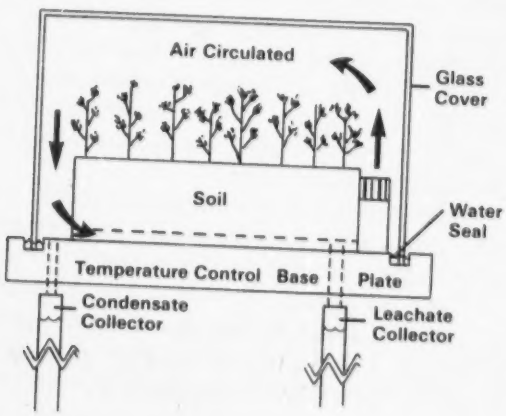

Fig. 1. Exposure chamber schematic.

area. In the dark, relative humidity was 60% and the water flow was 0.5 ml min^{-1} m^{-2}. The CO_2 concentration was maintained at 350 ppm and in the light net-CO_2 assimilation was typically about 600 μmoles CO_2 min^{-1} m^{-2}. Plant and soil respiration was measured in the dark by monitoring the increase in CO_2 concentration and was generally about 250 μmoles CO_2 min^{-1} m^{-2}. The stability of these parameters over time was used to indicate organism metabolic consistency. The photoperiod was 16 hours light/8 hours dark and the photosynthetic photon flux density (PPFD) was 500 μE m^{-2} s^{-1} using 80% cool white fluorescent 1500 mA lamps and 20% incandescent lamps. Air temperature was 25 ± 1°C and at night was 20 ± 1°C.

Experiments were conducted with soil alone, a Calico fine sandy loam, and with the soil planted with bermuda grass *(Cynadon dactylon)*, or alfalfa *(Medicago sativa)*. These plants were grown in 25-cm-deep stainless steel trays in the glass house prior to transferring to the exposure chambers for acclimation and subsequent exposure to benzene. The alfalfa and bermuda grass were mature plants, having grown through several cuttings and were trimmed to 10 cm high at the time the tests were started.

After benzene injection, vapor concentrations were monitored by FID/GC. Measured gas samples were also purged, first through liquid scintillation cocktail (LSC), then through LSC containing a CO_2 gathering compound (Carbosorb). In the first solution, nonpolar vapors were solubilized and sequestered for ^{14}C counting. In the second solution, $^{14}CO_2$ which passed through the first solution was collected and

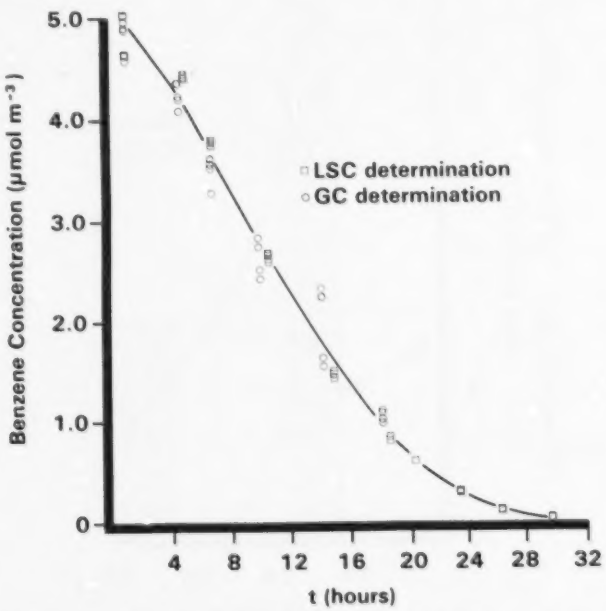

Fig. 2. Benzene concentration in the air of an exposure chamber containing bermuda grass. Measurements were made by gas chromatography and by ^{14}C analysis.

analyzed. Soil leachate and transpired water were analyzed for ^{14}C content by adding water directly to LSC and counting. In addition, soil and plant samples were oxidized to CO_2 and analyzed for ^{14}C content. Soils were also analyzed for degradation products by a series of extractions and determinations (Cross, 1979).

3. Results and Discussion

Within the environment of the exposure chambers, benzene concentrations decreased rapidly as presented in Figure 2. The experimental concentration of 5 μmol m^{-3} (0.11 ppm v/v at standard temperature and pressure) was selected because it is representative of often encountered ambient levels. After 12 to 16 hours, the air concentrations of benzene were no longer detectable by FID/GC. Analysis of the air by liquid scintillation techniques (LSC) using the previously described scrubbers indicated ^{14}C-labeled benzene depletion rates similar to those obtained by GC analysis. This observation revealed information regarding the nature of the reaction responsible for benzene degradation. The fact that the curves were similar implies that no volatile, nonpolar degradation products were formed. Such products would have been sequestered in the liquid scintillation cocktail, causing a different depletion pattern. Also,

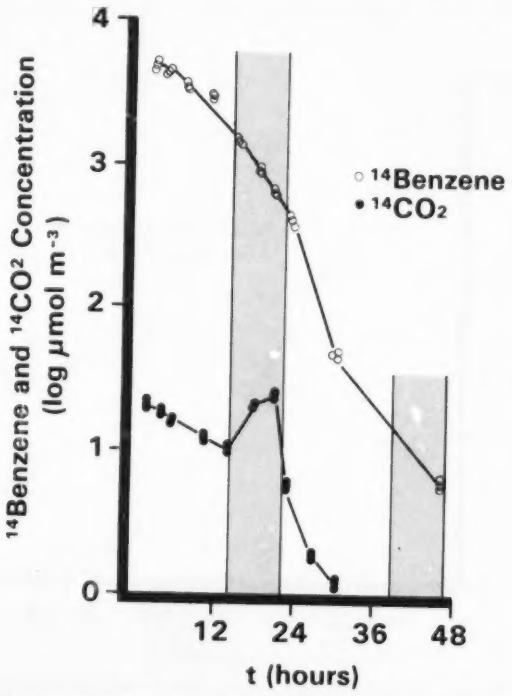

Fig. 3. Atmospheric concentrations of ^{14}C-benzene and $^{14}CO_2$.

they would probably have shown up on the chromatographs as identifiable peaks.

A measurable concentration of $^{14}CO_2$ appeared in the air in the first sampling and then decreased over the next 2 days. An example is shown in Figure 3. In the light, the $^{14}CO_2$ concentration curve must be conceptualized as the result of several simultaneous reactions. Labelled carbon dioxide is generated from the bacterial degradation of benzene. It may be absorbed in soil-water to form carbonate, or it may diffuse to the soil surface, mix with air, and become part of the CO_2 assimilated by plants. Since the CO_2 assimilation rate was known, a simple ratio of that rate to the $^{14}CO_2$ air concentration yielded the $^{14}CO_2$ evolution rate. In the dark, the slope of the increasing $^{14}CO_2$ concentration yielded a similar evolution rate. When the rate of the $^{14}CO_2$ evolution was compared to the rate of atmospheric benzene depletion, a constant ratio did not result, but rather an increasing ratio with time. This implies that the sinking of benzene to the soil was mediated by a reaction which occurred at a rate independent of the final benzene degradation process. It is presumed that the decomposition of benzene occurred in a series of finite metabolic oxidation steps resulting in the elimination of carbons from the organic moiety in the form of CO_2. Within the dynamics of the degradation of the benzene molecule is a rate limiting step. This reaction is the controlling factor in the observation of the formation of CO_2 from benzene, i.e., within the series:

$$C_6H_6 \xrightarrow{k_1} C_5 \text{ Product} + CO_2$$

$$C_5 \text{ Product} \xrightarrow{k_2} C_4 \text{ Product} + CO_2$$

$$C_4 \text{ Product} \xrightarrow{k_3} C_3 \text{ Product} + CO_2$$

etc.

The rate constant which reflects the slowest reaction will be reflected in the observation of the formation of $^{14}CO_2$. On the other hand, the observation of the decrease of benzene from the atmosphere reflects the rate of production of the C_5 product via rate constant k_1.

Atmospheric benzene depletion curves were found to adhere to first order kinetics. Thus, the calculations of rate constants are based on

$$C_t = C_0 e^{-kt}$$

where C_t is the concentration of atmospheric benzene at any time, t, C_0 is the concentration of benzene at $t = 0$, and k is the first-order rate constant. The rate constants were determined by calculating the slope of $\ln C_t/C_0$ vs t using plots as shown in Figure 4. The slopes and linear fit analysis are presented in Table I. The rate constants for benzene depletion from the atmosphere indicates that little difference was observed for the two plant species tested. However, the difference between exposure chambers containing plants and soil and those chambers containing only soils was found to be significant.

In later experiments the stems and leaves of alfalfa and bermuda plants were cut immediately below the soil surface and the plant material was carefully removed from

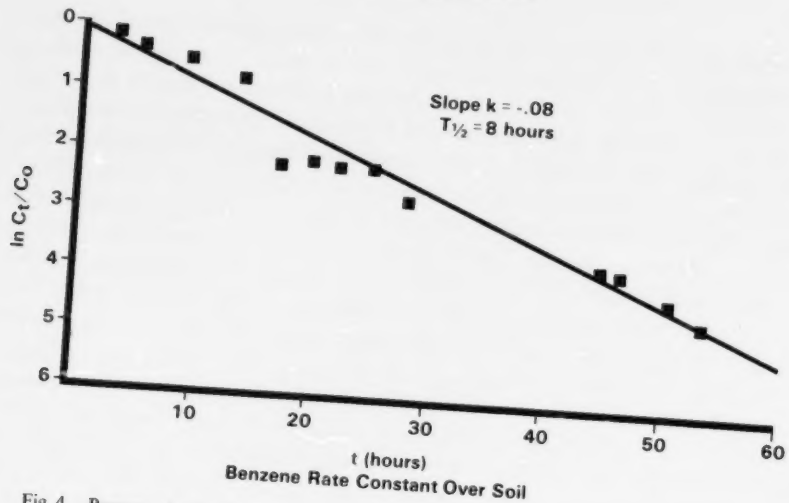

Fig. 4. Benzene depletion rate constant determined for transfer from air to unplanted soil.

the chambers. The plants were placed in empty chambers and all chambers were dosed with benzene. As indicated in Table I, no significant difference was observed by removing the aerial plant organs. In addition, no appreciable activity was observed in the chambers containing only plant material.

The soils were then sterilized by repeated autoclaving. After replacement in the chambers, they were dosed with benzene and the observations repeated. No depletion of atmospheric benzene was observed.

TABLE I

Observed rate constants.

Chamber Contents	Rate Constants (K)				
	Condition Normal	R^2	Plants Removed	R^2	Sterilized
Bermuda in Soil	0.15	0.98	0.17	0.97	0.00
Alfalfa in Soil	0.14	1.00	0.16	0.99	0.00
Soil Only	0.08	0.97	0.08	0.98	0.00
Cut Plants Only	NA		0.00		NA

The loss of benzene observed from the atmosphere in terrestrial exposure chambers suggests that a natural environmental sink exists for benzene vapors. The effective field rates will certainly depend on soil temperature, moisture, porosity, the endogenous population of appropriate organisms, air turbulence, resistance to air contact with the soil and probably several other variables. Therefore the rate constants generated by these experiments are not expected to fit field conditions exactly; however, they are

useful as a first approximation and represent the most probable route of atmospheric benzene movement to a decontaminating sink.

In the planted exposure chambers (bermuda grass and alfalfa) the benzene depletion rates were more rapid both with and without the foliage than in unplanted soil. Most probably a more active microorganism population in the planted soil accounted for this difference. Benzene depletion half times of 4.6, 5.0, and 8.0 hours for bermuda, alfalfa, and unplanted soil, respectively, were calculated. These rates are sufficient to indicate that soil microorganisms represent a major environmental depletion pathway for benzene.

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